Review Article

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Antithrombotic actions of statins

Andrew Szczeklik, Anetta Undas, Jacek Musial, Piotr Gajewski, Jakub Swadzba, Milosz Jankowski

Department of Medicine, Jagiellonian University School of Medicine, Cracow, Poland

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SUMMARY

Aspirin depresses thrombin generation, probably through a mechanism independent of the cyclooxygenase inhibition, but rather related to acetylation of the platelet membrane macromolecules. This action of aspirin is blunted in hypercholesterolemia. In men with marked hypercholesterolemia, lowering serum cholesterol by a three-month simvastatin treatment is accompanied by a reduction of thrombin generation both at basal conditions in venous blood and after activation of hemostasis by microvascular injury. Similar results are obtained in patients with coronary heart disease and borderline – high cholesterol levels. We assessed tissue-factor initiated coagulation in blood samples collected every 30-seconds from bleeding time wounds in patients with advanced coronary artery disease and total cholesterol levels of 224 mg/dL. Three-month simvastatin treatment depressed blood clotting, leading to reduced rates of prothrombin activation, FVa generation, fibrinogen cleavage, FXIII activation, and an increased rate of FVa inactivation. Such a concerted influence of statins on the clotting cascade seems to be independent of their lipid-lowering action and may be the result of depressed isoprenoid production.

Clinical trials demonstrated effectiveness of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, or statins, in primary and secondary prevention of coronary artery disease (CAD). In most reports, these drugs exerted stronger beneficial effects on coronary endpoints than those anticipated from analysis of the reduction in total cholesterol (TC) and LDL cholesterol levels [1]. Over the last years, evidence has accumulated that inhibition of HMG-CoA reductase result in pleiotropic, antiatherothrombotic effects, often attributed to the inhibition of isoprenoid production secondary to inhibition of mevalonic acid synthesis [2,3]. These properties, which appear to be independent of their lipid-lowering activity, include: stabilization of atherosclerotic plaque [4], suppression of inflammation [5], impairment of vascular smooth muscle cell proliferation [6], promotion of blood vessel growth [7], improved endothelial function through nitric oxide (NO)-dependent mechanisms [8], stimulation of fibrinolysis [9], and poorly described modulation of blood coagulation [3]. Since statins dramatically decrease the incidence of acute coronary events and ischemic stroke and the most critical aspect of atherosclerosis from clinical point of view is plaque rupture and thrombus formation in loco, antithrombotic alterations in the blood coagulation cascade following statin therapy could be expected. Indeed, several studies, performed mainly in vitro, suggested that statins can influence directly or indirectly coagulant functions of platelets, blood cells, vascular walls, circulating coagulation factors or cofactors.

Until now, there is great controversy about effects of statins on platelet function [10,11]. Available data suggest that statins inhibit platelet-mediated thrombin generation and its proteolytic activity. A marked decrease in thrombin generation, as evidenced by reductions in plasma thrombin generation markers, such as prothrombin fragment (F1+2), fibrinopeptide A (FPA) and thrombin-anti-

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Correspondence address: Andrew Szczeklik MD PhD, Department of Medicine, Collegium Medicum, Jagiellonian University, ul. Skawinska 8, 31-066 Cracow, Poland, e-mail: mmszczek@cyf-kr.edu.pl

thrombin III complexes (TAT), has been reported in hypercholesterolemic subjects treated with pravastatin or simvastatin [12,13]. Some authors showed a correlation between the magnitude of reduction in thrombin formation or alteration in platelet function and hypolipemic effect of statins [14,15]. The nature and characteristics of statin-induced thrombin-lowering effects are still elusive.

Here, we summarize results of our studies on antithrombotic properties of simvastatin, a potent lipophylic statin.

We observed that aspirin's antithrombotic action was blunted in hypercholesterolemic subjects [16], we decided to investigate the impact of marked reduction in TC on this phenomenon. To address this important issue, we have evaluated the TF-initiated coagulation at the site of hemostatic plug formation, in blood obtained from bleeding-time wounds [17]. Since the kinetics of coagulant reactions in vivo cannot be extrapolated from static systems, we have chosen a model of microvascular injury, which offers a unique approach to the physiological situation when the damaged vessel wall and subendothelium are suddenly exposed to circulating blood [18]. We hypothesize that alterations in the blood coagulation cascade and/or platelet function, providing catalytic surface for prothrombinase formation should be evident in the in vivo model. When factor (F)VIIa encounters tissue factor (TF) exposed at the site of injury, they form the extrinsic tenase, which activates FX and FIX to their active forms, and then, FXa assembles with FVa into the prothrombinase complex, converting prothrombin to thrombin. This multifunctional enzyme activates cofactors FV and FVIII, thereby amplifying blood coagulation [19].

We treated 33 symptom-free men with markedly elevated TC levels (mean, 8.03 mmol/L) and LDL cholesterol (mean, 5.59 mmol/L) for 12 weeks with simvastatin (20–40 mg/d), followed by an additional two-week combined therapy with the statin and aspirin (300 mg/d). An approximate production of thrombin within the first 180 seconds of bleeding was estimated on the basis of the area under the concentration curve of F1+2 and FPA values in 30-second intervals. A 3-month simvastatin therapy resulted in a significant decrease in TC by 31% and LDL-cholesterol by 42%. At sites of microvascular injury, thrombin generation became markedly depressed by simvastatin, as evidenced by reduced initial thrombin concentration and total amount of the enzyme generated. In venous blood, only FPA levels were decreased. Addition of aspirin to the statin did not lower thrombin formation in the model used or venous blood. We, therefore, concluded that reduction in blood cholesterol concentrations is not able to restore the inhibitory effect of aspirin on thrombin generation to its magnitude, observed by us previously in normocholesterolemic subjects [20].

These observations in patients with marked hypercholesterolemia prompted a further study in CAD patients with average cholesterol levels, treated with low-dose aspirin [21]. In 25 male patients with stable CAD, with a mean age of 55 years, and TC levels below 6.5 mmol/L, F1+2 concentrations were measured in bleeding time blood every 30 seconds. A 3-month simvastatin treatment led a decrease in the total amount of prothrombin converted to thrombin by about 50%. This reduction was accompanied by a significant decrease in TC from 5.9 to 4.4 mmol/L and LDL cholesterol from 3.7 to 2.2 mmol/L. These reductions did not correlate with the magnitude of thrombin-lowering action of simvastatin. Moreover, a significant prolongation of bleeding time following simvastatin (by 38 s) has been found. We clearly demonstrated that thrombin-lowering action of simvastatin are not limited to marked hypercholesterolemia but extend to patients with slightly elevated blood cholesterol levels.

In the light of studies showing reduced thrombin formation following statin therapy, we put forward a hypothesis that statins may alter FV, FXIII, and prothrombin activation as well as FVa inactivation by activated protein C (APC). To provide a comprehensive view of simvastatin-induced anticoagulant effects, we studied time-courses of coagulant reactions pertinent to efficient blood clotting prior to and after a 3-month simvastatin treatment (20 mg/d). The study population comprised 17 men, aged 39-64 (mean, 51.6) years, with documented advanced coronary artery disease and/or stable angina and with average serum TC (mean, 5.8 mmol/L) and LDL cholesterol (mean, 3.6 mmol/L) levels [22]. Quantitative Western blotting was performed, using antibodies against fibrinogen, FV heavy and light chains, the subunit A of FXIII and prethrombin 1, which recognizes, among other, prothrombin and thrombin B-chain [23].

Treatment with simvastatin led to significant reductions in both TC (from 5.8 mmol/L to 4.5 mmol/L) and LDL cholesterol (from 3.6 mmol/L to 2.3 mmol/L), while triglycerides and HDL-C did not al-

ter. Interestingly, bleeding time became slightly, but significantly, prolonged by 37 s. Administration of simvastatin was associated with potentially favorable alterations in blood coagulation, including statistically significant decreases in maximum rates of:

(1) activation of prothrombin (by 13%) and formation of α-thrombin B-chain (by 25%),
(2) generation of FVa heavy chain (by 30%) and FVa light chain (by 17%),
(3) fibrinogen conversion to fibrin (by 64%),
(4) factor XIII activation (by 20%).

Changes in the kinetics of all the reactions studied were accompanied by a significant delay by 30–60 s.

Fibrinopeptide A (FPA) and B (FPB) levels in blood collected within the last 30 seconds of bleeding were determined by high pressure liquid chromatography (HPLC), and their identification confirmed using matrix-assisted laser desorption ionization-time flight (MALDI-TOF) mass spectrometry. Following simvastatin treatment FPA concentration decreased from 4 μmol/l to 3 μmol/l, whereas levels of FPB, cleaved slowly from the B chains of fibrinogen, fell from 0.8 μmol/l to levels below detection limit. This finding confirmed impairment of thrombin-mediated proteolysis of fibrinogen, as a result of simvastatin therapy.

Unexpectedly, inactivation of FVa was affected by simvastatin treatment. There was a significant acceleration of a release of the 30 kD fragment of FVa heavy chain (residues 307-506), a product of FVa cleavage by APC. This fragment appeared faster – by 90 s – after treatment with simvastatin. We concluded that simvastatin treatment may modulate the major anticoagulant system in humans, the protein C pathway, which inactivates FVa and FVIIIa and shuts down thrombin formation [24]. In the microvasculature, where thrombomodulin concentration is high relative to the blood volume, FVa degradation occurred much faster than during clotting of the whole blood [23]. Since thrombin generation was impaired by simvastatin treatment, a faster appearance of the 30 kD fragment of FVa heavy chain is unlikely to result from an increase in thrombin-mediated protein C activation. It might be speculated that more rapid FVa inactivation following simvastatin treatment may be related to alterations in thrombomodulin expression on the endothelial cells or its cofactor function. It cannot be excluded that statin-induced impairment of platelet function decreases the amount of thrombomodulin released in loco [25]. Acceleration of APC-mediated inactivation of a cofactor of prothrombinase, FVa, is likely to contribute largely to antithrombotic actions of simvastatin in vivo.

Another important issue is whether antithrombotic actions of statins are related to their cholesterol-lowering effects. Results of in vitro studies suggest that atherogenic lipoproteins could enhance thrombin generation by promoting formation of the prothrombinase complex [26], however, triglycerides are probably more potent in providing additional surface for the assembly of prothrombinase [27]. Our data strongly argue against any relationship between hypolipemic and antithrombotic actions of simvastatin because reductions in thrombin generation or FVa formation showed no relationship with the initial lipid profile and the degree of serum cholesterol reduction.

It remains to be determined which mechanisms produce a shift toward anticoagulation in the hemostatic balance following a 3-month simvastatin treatment. Suppression of TF exposure appears to be a plausible explanation. Retarded and slower thrombin-mediated FV and FXIII activation appear to be secondary to posttreatment decrease in thrombin production. Prolongation of a lag phase in thrombin generation, which is determined by a concentration of the initiator of the extrinsic coagulation pathway, the complex TF-FVIIa [28], also suggests statin-induced downregulation of TF expression in the endothelium and subendothelium. Until now, decreased TF expression, induced by statins, has been, however, demonstrated solely on cultured human macrophages or monocytes. Tissue factor-induced formation of thrombin on human monocytes, stimulated by lipopolisacharydes, was significantly decreased by simvastatin at final concentrations of 10 nmol/l to 10 μmol/l [29].

A postulated mechanism by which simvastatin might change the coagulation reactions is inhibition of the synthesis of isoprenoids such as farnesyl and geranylgeranyl pyrophosphates, which are substrates for posttranslational modification – isoprenylation – of numerous intracellular proteins, including GTP-binding proteins [30]. Suppression of isoprenoid production by statins may lead to decreased expression of TF in the endothelium and/or subendothelium. Decreased TF expression could also be due to statin-induced upregulation of endothelial NO synthase [31], leading to an increase in NO production, which depresses TF expression in the endothelium [32]. Decreased tumor necrosis factor (TNF-α) expression by macrophages, which has

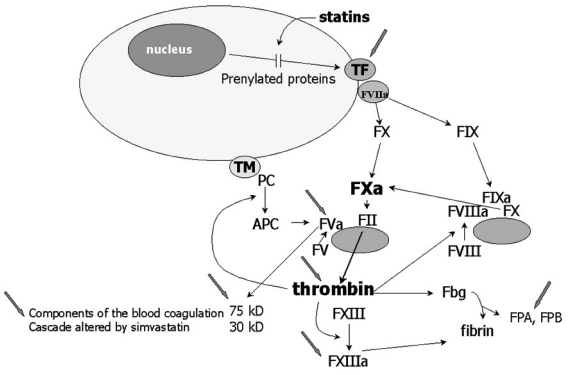

Figure 1. Postulated mechanisms of anticoagulant properties of simvastatin. Abbreviations used – see the text.

been reported in patients with polygenic hypercholesterolemia after eight weeks of simvastatin treatment [33], can provide a link between antithrombotic and anti-inflammatory actions of statins, because it is well known that pro-inflammatory cytokines increase the expression of TF.

Fenton et al. [34] attempted to form a coherent theory based on available data and postulated that decreased TF expression, combined with the downregulation in cell signaling following thrombin activation of protease activated receptor-1 (PAR-1), may explain antithrombotic properties of HMG--CoA reductase inhibitors. They proposed that statins may constitute a new class of antithrombotic drugs, possibly effective in patients with a prothrombotic tendency of another than atherosclerotic origin. It should be mentioned that very recently statins have been found to decrease FVII coagulant activity in hyperlipidemic patients [35]. A concept of reduced formation of the TF-FVIIa complexes in response to statin therapy deserves further studies.

Important remaining issues concerning antithrombotic effects of statins pertain to potential differences between different agents of this class, contribution of concomitant diseases or coronary risk factors, including high cholesterol levels, to their presence and to dose-dependance of these actions. First, there is no reason to suspect that actions of pravastatin, atorvastatin or cerivastatin differ from these observed during treatment with moderate doses of simvastatin. However, some studies suggested a differential effect of statins on nonlipid properties. For example, all statins, except pravastatin, decreased proliferation of arterial myocytes. The hydrophilicity of pravastatin could be implicated in this lack of action due to impaired diffusion of the drug into extrahepatic cells. Second, chronic metabolic disorders, such as diabetes, might alter, if not even abolish, antithrombotic actions of statins. Moreover, it is reasonable to hypothesize that since these effects are not related to lowering of cholesterol, also patients with marked hypercholesterolemia are likely to benefit from statins. The same could be true for normolipemic subjects and gives impetus to studies on the impact of statin treatment in other disorders associated with procoagulant tendency, for example, autoimmune diseases. Finally, it could be expected that the higher dose of statin, the more profound are anticoagulant alterations in the blood coagulation cascade.

In summary, statins can impair blood coagulation at the site of hemostatic plug formation in CAD patients with hypercholesterolemia. Simvastatin treatment results in a reduced rate of fibrinogen cleavage, FVa generation, and both prothrombin and FXIII activation and also by faster FVa inactivation (Fig. 1). Our studies support the concept that neither the initial lipid profile nor the magnitude of LDL cholesterol and/or TC reduction after simvastatin treatment correlate with maximum rates of the coagulant reactions at sites of hemostatic plug formation. It is well known that the thrombogenic response to plaque disruption depends on circulating hemostatic factors, local blood blood conditions, and interactions between blood and the vessel wall. Hence, we believe that antithrombotic effects here discussed have vital therapeutic implications and account, at least in part, for the unquestionable clinical benefits, observed during treatment with statins.

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